

Research Note

Effect of Distillers Feedstuffs and Lasalocid on *Campylobacter* Carriage in Feedlot Cattle

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ABSTRACT

Campylobacter bacteria are foodborne pathogens that can colonize the gut of food animals. Limited in their ability to ferment sugars, *Campylobacter* can derive energy for growth via amino acid catabolism. The objectives of the present studies were to test whether supplemental distillers grains containing high amounts of rumen-undegradable intake protein or supplemental lasalocid may, by promoting amino acid flow to the lower bovine gut, increase intestinal carriage of *Campylobacter*. In study one, 10 steers (5 per treatment) were adapted to diets formulated to achieve 0 or 30% dried distillers grains. After an initial 14-day adaptation to the basal diet, control and treated steers were fed their respective diets for 23 days, after which time they were fed supplemental lasalocid for an additional 8 days, followed by a 5-day withdrawal. In study two, 24 steers preacclimated to a basal diet were adapted via 3-day periodic increases to dietary treatments formulated to achieve 0, 30, or 60% wet corn distillers grains with solubles. Analysis of *Campylobacter* bacteria cultured from duodenal and fecal samples in study one and from fecal samples in study two revealed no effect of dried distillers grains or wet corn distillers grains with solubles on the prevalence or concentrations of duodenal or fecal *Campylobacter*. The results from study one indicated that colonized steers, regardless of treatment, harbored higher *Campylobacter* concentrations when transitioned to the basal diet than when coming off pasture. *Campylobacter* carriage was unaffected by lasalocid. These results provide no evidence that feeding distillers grains high in rumen-undegradable intake protein or supplemental lasalocid contributes to increased intestinal carriage of *Campylobacter* in fed cattle.

Campylobacter bacteria are a leading bacterial cause of human foodborne illness throughout the world. According to the Centers for Disease Control and Prevention Web site (5), human campylobacteriosis typically manifests as a painful and serious diarrheal disease, sometimes accompanied by nausea, vomiting, and systemic infection, the latter being of critical importance in immunocompromised individuals. *Campylobacter* infections can also result in postinfection immune-mediated neuropathies, such as Guillain-Barré syndrome or Miller Fisher syndrome (13, 15). In the United States, 14 human infections per every 100,000 individuals in the population were estimated to occur during the year 2012, which represents a 14%

increase over the yearly incidence estimates from 2006 to 2008 (6).

Contaminated unpasteurized dairy products and improperly cooked poultry products are recognized as major sources of foodborne outbreaks; however, other foods may also exist as a source of contamination (5, 18). Associations between human cases of campylobacteriosis and the consumption of beef products with findings of high carriage rates of *Campylobacter jejuni* in the bovine gastrointestinal tract were reported (1, 2, 7, 22). The prevalence of *C. jejuni* has been reported to be higher in feedlot cattle (58 and 30%) than in pastured cattle (2 and 5%) (1, 7), and the prevalence increased in fed cattle from 1.6% near or upon entry to the feedlot to 63% near the end of the finishing period (2). This latter finding was attributed to an epidemiological spread of the organism from a few colonized animals to many within the more densely populated rearing conditions within the feedlot; however, other factors, such as diet or animal husbandry practices, could potentially contribute to this increase. More recently, Gutierrez-Bañuelos et al. (9) reported that steers fed diets supplemented with tannin extracts shed approximately 10- to 100-fold more *Campylo-*

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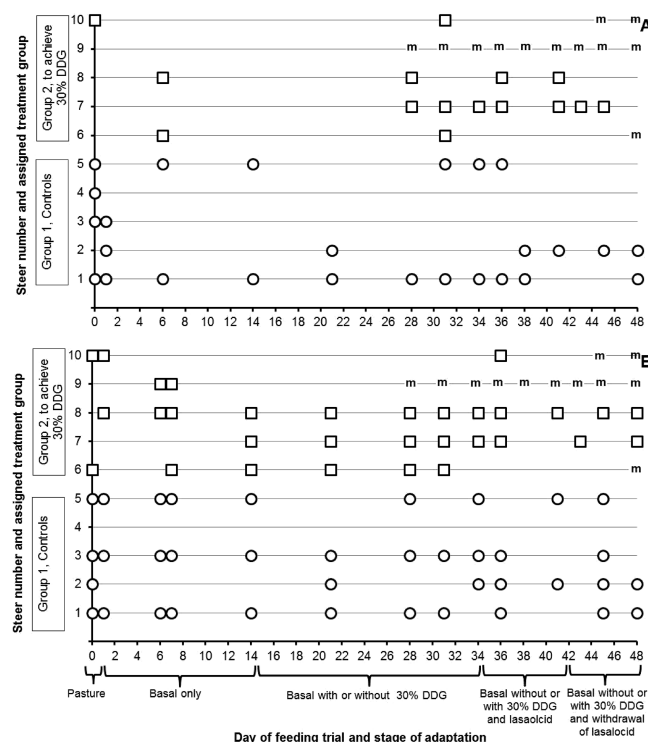


FIGURE 1. Qualitative isolation of *Campylobacter* from duodenal (A) and fecal (B) contents of steers transitioned as described on the x axes to diets supplemented to achieve 0% (group 1, controls) or 30% (group 2, to achieve 30% DDG) dried distillers grains. The occurrence of positive *Campylobacter* isolations from individual steers within each group (identified on the y axes) over the course of the 48-day study is denoted by circles for steers assigned to group 1 and squares for steers assigned to group 2; m denotes missing data due to unavailability of samples.

bacter organisms within their feces than unsupplemented control steers. Considering that tannins are recognized for their ability to protect dietary protein from ruminal degradation, we hypothesized that the tannin treatments may have increased the intestinal availability of amino acid substrates potentially utilized by asaccharolytic, amino acid-fermenting *Campylobacter* bacteria (12, 20, 21).

The practical question resulting from this hypothesis is whether or not diets supplemented with rumen-undegradable protein sources intended to deliver more amino acids for intestinal absorption unintentionally promote increased intestinal growth and subsequent shedding of amino acid-utilizing *Campylobacter*. Likewise, ionophores like monensin and lasalocid are often supplemented to ruminant diets to protect proteins from being catabolized by obligate amino acid-fermenting bacteria that play a major role in ruminal amino acid degradation (3). Could the inhibition of these competing amino acid-utilizing bacteria by ionophores allow *Campylobacter* unrestricted growth in the lower gut? The primary objectives of this study were to determine the effects of feeding undegradable intake protein and supplemental lasalocid on *Campylobacter* colonization and shedding in feedlot cattle.

MATERIALS AND METHODS

In our first experiment, 10 Angus steers averaging 434 kg (± 41 standard deviation [SD]) and each surgically implanted in a

prior study with duodenal and ruminal cannula were brought in from pasture (predominantly Coastal bermudagrass) and randomly allocated ($n = 5$ per diet) to diets formulated to achieve 0 or 30% dried distillers grains (DDG; dry matter basis). Experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at Texas A&M University. Steers were housed in individual pens (2.1 by 1.5 m). Beginning on day 1 of the study and continuing until the end of the study on day 48, steers assigned to the 0% DDG diet (0DDG) were fed a basal diet containing cracked corn, supplemental fat, cottonseed meal, rice bran, and minerals and vitamins to achieve maintenance requirements (14). Steers assigned to the 30% DDG diet (30DDG) were fed the basal diet for 14 days and were then switched to the diet containing 30DDG in place of corn, which was fed for the remainder of the study. Lasalocid (Bovatec, Zoetis, Florham Park, NJ) was fed according to the directions on the label to steers assigned to both 0DDG and 30DDG diets on day 36 and continuing to day 43 of the study to investigate the potential protein-sparing effect of this ionophore on *Campylobacter* colonization. Beginning on day 44 and continuing to the end of the study on day 48, the steers in both treatment groups were again fed their assigned 0DDG or 30DDG diets without lasalocid supplementation.

Ruminal, duodenal, and fecal samples were collected on day 0 (8 April 2009), which represented gut samples from cattle having grazed for >2 weeks on Coastal bermudagrass pasture, and on days 1, 6, 7, and 14 during the initial 2 weeks of adaptation to the basal diet. Thereafter until day 34, samples were collected on days 21, 28, and 34 from steers assigned to the basal diet (0DDG) and from steers assigned to the 30DDG diet, which commenced feeding on day 15. Samples were collected on days 36, 38, 41, and 43 during the lasalocid feeding period, which began on day 36 and ceased after the last meal on day 43. The final sample collections were taken on days 45 and 48, which represent 2 and 5 days after the cessation of lasalocid feeding. Three steers were removed from the 30DDG diet because of leaking duodenal cannula, the first being removed on day 28 of the study, the second on day 43, and the remaining steer on day 48. All samples were collected just before the morning feeding (8:00 a.m.) and returned to the laboratory within 2 h of collection. Upon arrival at the laboratory, 1 g of each sample was serially diluted (10-fold) in phosphate buffer (pH 6.8) and plated onto Campy Cefex agar (17) for viable cell count enumeration of *Campylobacter* as routinely done in our laboratory (11). Colonies were counted after 48 h of microaerobic (N_2 - CO_2 - O_2 , 85:10:5) incubation at 42°C.

In our second study, crossbred steers averaging 385 kg (± 29) were blocked by body weight and randomly assigned (8 steers per diet) to diets including wet corn distillers grains with solubles (WDGS; dry matter basis) formulated to achieve 0% (0WDGS), 30% (30WDGS), or 60% (60WDGS) WDGS. These steers were part of a group of animals also used to examine rumen microbial diversity in individually fed steers, and the results, as well as specifics related to diet formulation, treatment allocation, and animal care and use approval by West Texas A&M Institutional Care and Use Committee, were reported previously (19). Briefly, steers were acclimated to their pens and trained to use Calan gates (American Calan, Northwood, NH), enabling each steer exclusive access to their own individual diet beginning at least 2 weeks before the study began and until the end of the study. All steers received the same basal diet containing 0% added WDGS during this acclimation period. Steers receiving WDGS were transitioned via incremental increases (every 3 days) to diets containing 15, 30, 45, or 60% WDGS, which replaced steam-flaked corn, supplemental fat, and cottonseed meal in the basal diet. Fecal samples were collected via rectal palpation. Upon sampling on day 0 (5

TABLE 1. Overall mean *Campylobacter* concentrations in duodenal contents and feces of steers transitioned to diets supplemented to achieve 0 or 30% dried distillers grains

Period, groups, and diet	Overall <i>Campylobacter</i> concn, mean log CFU/g \pm SD (no. of steers) ^a				
	Duodenal contents			Feces	
	Group 1	Group 2	Combined	Group 1	Group 2
Period 1 (day 0)					
Groups 1 and 2 coming off pasture	2.27 \pm 0.81 (5)	1.44 \pm 0.31 (5)	1.86 \pm 0.72 (10)	2.46 \pm 0.73 (5)	1.64 \pm 0.48 (5)
Period 2 (days 1, 6, 7, 14)					
Groups 1 and 2 transitioning to basal	1.97 \pm 1.02 (20)	1.51 \pm 0.73 (20)	1.74 \pm 0.91 (40)	2.01 \pm 0.74 (20)	1.80 \pm 0.66 (20)
Period 3 (days 21, 28, 31, 34)					
Group 1 on basal; group 2 transitioning to 30DDG	2.21 \pm 1.45 (20)	2.37 \pm 1.54 (17)	2.28 \pm 1.47 (37)	2.23 \pm 0.94 (20)	2.63 \pm 1.28 (17)
Period 4 (days 36, 38, 41)					
Group 1 on basal; group 2 on 30DDG; both with lasalocid	2.17 \pm 1.50 (15)	2.15 \pm 1.28 (12)	2.16 \pm 1.38 (27)	2.14 \pm 1.16 (15)	2.17 \pm 0.93 (12)
Period 6 (days 43, 45, 48)					
Group 1 on basal; group 2 on 30DDG; both without lasalocid	1.91 \pm 1.28 (15)	2.04 \pm 1.39 (8)	1.96 \pm 1.29 (23)	2.16 \pm 1.09 (15)	2.21 \pm 0.99 (8)
Period effect	0.9404	0.2033	0. 3788	0.8944	0.0908
Group effect		0.3989			0.5522
Interaction		0.6836			0.4208

^a Overall means were calculated to include *Campylobacter* culture-negative specimens assigned a limit of detection value of 1.30 log CFU/g.

TABLE 2. Subset mean *Campylobacter* concentrations in duodenal contents and feces of steers transitioned to diets supplemented to achieve 0 or 30% dried distillers grains

Subset <i>Campylobacter</i> concn, mean log CFU/g ± SD (no. of steers) ^a						
Period, groups, and diet	Duodenal contents			Feces		
	Group 1	Group 2	Combined	Group 1	Group 2	Combined
Period 1 (day 0)						
Groups 1 and 2 coming off pasture	2.52 ± 0.69 (4)	2.00 ± NA ^b (1)	2.41 B ± 0.64 (5)	2.76 BC ± 0.37 (4)	2.15 B ± 0.21 (2)	2.55 B ± 0.44 (6)
Period 2 (days 1, 6, 7, 14)						
Groups 1 and 2 transitioning to basal	3.22 ± 0.71 (7)	3.39 ± 1.54 (2)	3.26 AB ± 0.82 (9)	2.60 C ± 0.47 (11)	2.40 B ± 0.53 (9)	2.50 B ± 0.49 (20)
Period 3 (days 21, 28, 31, 34)						
Group 1 on basal; group 2 transitioning to 30DDG	3.89 ± 1.24 (7)	4.32 ± 0.69 (6)	4.10 A ± 1.01 (13)	3.00 ABC ± 0.52 (11)	3.35 A ± 1.00 (11)	3.18 A ± 0.80 (22)
Period 4 (days 36, 38, 41)						
Group 1 on basal; group 2 on 30DDG; both with lasalocid	3.92 ± 1.46 (5)	3.84 ± 0.53 (4)	3.88 A ± 1.08 (9)	3.39 AB ± 0.81 (6)	3.03 AB ± 0.32 (6)	3.21 A ± 0.61 (12)
Period 6 (days 43, 45, 48)						
Group 1 on basal; group 2 on 30DDG; both without lasalocid	4.36 ± 0.47 (3)	4.28 ± 0.53 (2)	4.32 A ± 0.43 (5)	3.44 A ± 0.12 (6)	3.12 AB ± 0.27 (4)	3.31 A ± 0.24 (10)
Period effect	0.1380	0.1280	0.0275	0.0107	0.0341	0.0006
Group effect		0.9705			0.1833	
Interaction		0.9330			0.2965	

^a Subset means were calculated inclusive of *Campylobacter* culture-positive specimens only. Means within the same column with unlike letters (A, B, and/or C) differ significantly (*P* < 0.05).
^b NA, not applicable.

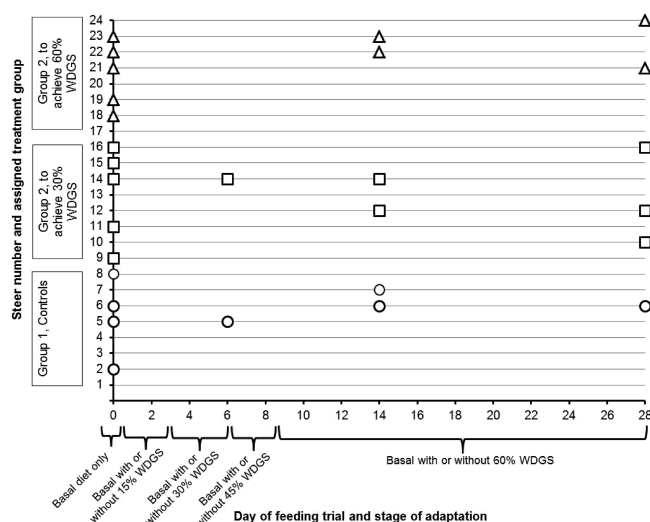


FIGURE 2. Qualitative isolation of *Campylobacter* from feces of steers transitioned as described on the x axis to diets supplemented to achieve 0% (group 1, controls), 30% (group 2, to achieve 30% WDGS), or 60% (group 3, to achieve 60% WDGS) wet dried distillers grains with solubles. The occurrence of positive *Campylobacter* isolations from individual steers within each group (identified on the y axis) over the course of the 28-day study is denoted by circles for steers assigned to group 1, by squares for steers assigned to group 2, and by triangles for steers assigned to group 3.

February 2009), all steers were still being fed the basal diet, but thereafter until the end of the 28-day feeding trial, only the 8 steers assigned to 0WDGS were fed the basal control diet. Thus, the controls had received the basal diet for all subsequent samplings (days 6, 14, and 28 of the study). Upon sampling on day 6, all 16 steers assigned to receive WDGS diets (30WDGS and 60WDGS) had just completed their third day of transition to 30WDGS; on all sampling days thereafter, steers assigned to 30WDGS were consuming a 30WDGS diet. Upon sampling days 14 and 28, the steers assigned to the 60WDGS diet had received their final diet for 2 and 16 days, respectively. On each day of sample collection, fecal samples were placed in individual Whirl-Pak bags (Nasco, Fort Atkinson, WI) that were closed and shipped overnight on ice to the Food and Feed Safety Research Unit laboratory in College Station, TX, where they were immediately processed for bacteriological enumeration of *Campylobacter* as described above.

Bacterial enumerations were transformed and expressed as log CFU per gram of duodenal or fecal content. For quantitative analysis of *Campylobacter* concentrations, samples yielding no detectable *Campylobacter* colonies were handled two different ways. The first was to assign these culture-negative samples a limit of detection value of 20 CFU/g of content (the equivalent of 1.30 log CFU/g) and include them in an analysis of overall mean *Campylobacter* concentrations. However, because the assignment of limit of detection values to culture-negative samples may overestimate *Campylobacter* concentrations in potentially *Campylobacter*-negative animals, a second analysis was also performed by excluding these culture-negative samples and using only the subset of samples having *Campylobacter* colonies above our limit of detection. Thus, the overall mean *Campylobacter* concentrations are inclusive of *Campylobacter* culture-negative specimens assigned a limit of detection value of 1.30 log CFU/g, whereas subset means reflect *Campylobacter* concentrations that are exclusive of samples yielding no observable colonies. Analysis

of overall *Campylobacter* concentrations was achieved by using a repeated-measures analysis of variance for the main effects of day, treatment group, or day \times treatment group interaction unless indicated otherwise. For these analyses, the day counts were also pooled within the feeding period and analyzed by a general analysis of variance for the main effects of period, treatment group, and period \times treatment group. For quantitative analysis of the subset *Campylobacter* concentrations, log-transformed counts were analyzed similarly except that tests for the effects of day and day \times treatment group interaction were omitted because of missing values on some days. For qualitative analysis, culture-negative samples yielding no observable colonies were compared with those yielding observable (culture-positive) *Campylobacter* colonies by using Fisher's exact test to look for period or group differences. All analyses were performed using Statistix9 Analytical Software (Tallahassee, FL).

RESULTS AND DISCUSSION

Detectable amounts of *Campylobacter* were only isolated from the ruminal contents of one steer in our first study, and the isolation was from contents collected on day 6 from a steer on 0DDG. This low frequency of isolation of *Campylobacter* from the rumen is not unexpected, as numerous reports have indicated that *Campylobacter* do not flourish in that environment (7, 9, 11, 16). The results from a qualitative analysis of the *Campylobacter* isolated from duodenal and fecal samples are presented in Figure 1 and reveal considerable daily variation in the proportions (expressed as percentages) of animals that were culture positive for *Campylobacter*. Fisher's exact test revealed no differences ($P > 0.05$) between the proportions of steers on 0DDG and 30DDG yielding *Campylobacter* culture-positive duodenal or fecal specimens on each day they were measured, with the proportions of culture-positive steers ranging from 0 to 100%. Similarly, significant differences between groups (ranging from 10 to 80%) or between experimental periods (ranging from 22 to 60%) were not observed when the proportions of culture-positive steers (Fig. 1) were combined and analyzed. For 0DDG steers, *Campylobacter* was isolated from duodenal contents most frequently (11 culture positive of 14 samples) from steer number 1 and then to a lesser extent from steers numbered 2 and 5 (each with 6 of 14 samples). For 30DDG steers, steer number 9 yielded no *Campylobacter* culture-positive duodenal contents but had to be removed from the study midway through its transition to the 30DDG diet. *Campylobacter* was isolated most frequently from steer 7 on 30DDG, with all isolations from this steer occurring midway or later during the transition to the 30DDG diet. Over the course of the entire study, *Campylobacter* was isolated more frequently ($P = 0.0008$) from fecal than from duodenal specimens and only steer number 4 on 0DDG was fecal-culture negative throughout all sampling periods. This was due mainly to significantly higher fecal isolations during periods 2 ($P = 0.0055$) and 3 ($P = 0.0309$), when the steers were transitioning to the basal feedlot diet with or without supplemental DDG (Fig. 1). This finding is consistent with the findings of Inglis et al. (10), who reported that *C. jejuni* preferentially colonizes by attaching to binding sites within the duodenum and jejunum of beef

TABLE 3. *Campylobacter* concentrations in feces of steers transitioned to diets supplemented to achieve 0, 30, or 60% wet dried distillers grains with solubles^a

Period 1	Period and diet	Day of study	Overall <i>Campylobacter</i> concn, mean log CFU/g ± SD (no. of steers)			Subset <i>Campylobacter</i> concn, mean log CFU/g ± SD of culture-positive samples only (no. of steers)		
			Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
After >2-wk acclimation to basal diet Combined for all steers on like diet ^b		0	2.88 ± 1.94 (8)	2.74 ± 1.28 (8) 2.82 ± 1.50 (24)	2.83 ± 1.41 (8)	4.46 ± 1.43 (3)	3.61 ± 0.59 (5) 3.90 ± 0.96 (14)	3.74 ± 0.83 (5)
Period 2		3	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Basal diet 3rd day on basal diet plus 15WDGS								
Period 3		6	1.51 ± 0.60 (8) — ^c	1.67 ± 1.06 (8) 1.49 ± 0.75 (16) ^d	1.30 ± 0.75 (8)	3.00 (1) —	4.30 (1)	None observed 4.30 (1) ^d
Basal diet 3rd day on basal diet plus 30WDGS								
Period 4		9	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Basal diet 6th day on basal diet plus 30DDG diet 3rd day on basal diet plus 45% DDG diet								
Period 5		14	2.04 ± 1.37 (8) — —	— 2.14 ± 1.56 (8) —	— — 2.09 ± 1.46 (8)	4.25 ± 0.35 (2) — —	— 4.66 ± 0.26 (2) —	— — 4.46 ± 0.02 (2)
Basal diet 11th day on basal diet plus 30WDGS 5th day on basal diet plus 60WDGS								
Period 6		28	2.00 ± 1.84 (8) — —	— 1.95 ± 0.90 (8) —	— — 2.39 ± 2.16 (8)	6.18 (1) — —	— 3.04 ± 0.04 (3) —	— — 5.65 ± 2.08 (2)
Basal diet 25th day on basal diet plus 30WDGS 19th day on basal diet plus 60WDGS								
Period effect				0.0146			0.5373	
Group effect				0.9966			0.2681	
Interaction				0.9923		Not analyzed due to too many missing values		

^a Overall means were calculated to include *Campylobacter* culture-negative specimens assigned a limit of detection value of 1.30 log CFU/g. Subset means were calculated inclusive of *Campylobacter* culture-positive specimens only.

^b Values are the means ± SD of groups 1, 2, and 3 values from animals on like diet.

^c —, there were no animals from this group on this diet.

^d Value is the mean ± SD of groups 2 and 3 values from animals on like diet.

cattle and, thus, would likely resist being in digesta voided through the duodenal cannula. Conversely, *Campylobacter* bacteria in more distal gastrointestinal sites were preferentially associated with luminal digesta (10), which would be expected to be more easily voided via excretion.

The results from our quantitative analysis of the concentrations of *Campylobacter* in our first study revealed neither an effect of period nor a period \times treatment group interaction ($P > 0.05$) on overall mean duodenal or fecal *Campylobacter* concentrations, which were inclusive of culture-negative specimens assigned a limit of detection value of 1.30 log CFU/g (Tables 1 and 2). Likewise, the overall mean concentrations of duodenal and fecal *Campylobacter* were not affected ($P > 0.05$) by day or by a day \times treatment group interaction (not shown). Conversely, a period effect but not a treatment group effect was observed on subset mean concentrations of duodenal and fecal *Campylobacter* inclusive only of those specimens from which *Campylobacter* were isolated (Tables 1 and 2). This latter finding indicates that these colonized steers, regardless of treatment, harbored higher concentrations of *Campylobacter* when transitioned to the basal diet (periods 2 or 3) than when coming off of pasture (period 1). Others have reported higher prevalences of cattle shedding *Campylobacter* under feedlot conditions (1, 2, 7). More recently, however, Grove-White et al. (8) reported that pasture grazing, particularly during the summer, was a risk factor promoting higher *Campylobacter* prevalence in dairy cattle than forage feeding. The overall or subset mean *Campylobacter* concentrations did not differ between periods 3 and 5, indicating that the addition of lasalocid to the diets and its subsequent removal had no effect on *Campylobacter* enumerated from either the duodenal contents or feces (Tables 1 and 2). It is important to note, however, that excluding culture-negative specimens from our subset mean calculations likely reflects an overestimation of *Campylobacter* counts, as potentially noncolonized animals, being culture negative, were excluded from the analysis. On the other hand, the assignment of limit of detection values of 1.3 log CFU/g to our overall mean concentrations likely marginalized potential differences.

In our second experiment, *Campylobacter* was isolated from the feces of 14 of the 24 steers upon assignment to their respective treatment groups and there was no difference in prevalence between groups (Fig. 2). Moreover, the overall mean and subset mean *Campylobacter* concentrations measured during period 1 (day 0) did not differ between treatment groups (Table 3), indicating no bias in random assignment of the steers to the respective groups. Only two steers had *Campylobacter* culture-positive fecal specimens during period 3 (day 6), with one being on 0WDGS, receiving the basal diet without supplemental DGG, and the other from the 30WDGS group, which along with the 60WDGS group at this period, was receiving the 30DDG-supplemented diet (Fig. 1). Thus, a Fischer's exact test between the control and treated groups on day 6 (period 3) revealed no difference ($P > 0.05$) in the proportions of *Campylobacter*-positive steers (1 of 8 in the 0WDGS group versus 1 of 16 in the 30WDGS and 60WDGS groups). However, compared with the

proportions of *Campylobacter* culture-positive steers measured in period 1 (day 0), at which time all steers were receiving the control diet, we observed significant reductions ($P = 0.0300$) in the proportions measured in period 3 (day 6) in both the controls (0WDGS) and steers fed the 30WDGS diet. Similarly, the proportions of steers yielding *Campylobacter* culture-positive fecal specimens were markedly lower, although not necessarily significantly so, on all subsequent sampling days or periods than the proportions measured in period 1 (Fig. 2). The lower prevalences observed on days 6, 14, and 28 relative to that observed on day 0 are not readily explained. It is possible that the prevalence had peaked on day 0, which was after all steers had been acclimated to the same basal diet during their training to use their individual Calan gate feed bunks.

With the majority of steers yielding *Campylobacter* culture-negative fecal specimens during periods 3, 5, and 6, the contribution of the assigned limit of detection values consequently marginalized potential differences between group overall mean *Campylobacter* concentrations, as these did not differ ($P = 0.9966$) by treatment group (Table 3). There was, however, a main effect of period ($P = 0.0146$), with the overall means being lower in period 3 (1.50 ± 0.69 log CFU/g) than in period 1 (2.82 ± 1.50 log CFU/g) and the overall means in periods 5 and 6 being intermediate (2.10 ± 1.40 and 2.12 ± 1.65 log CFU/g, respectively). As with our qualitative estimates of *Campylobacter* prevalence in these steers, it is possible that the *Campylobacter* concentrations had also peaked on day 0. An interaction between period and treatment group was not observed ($P = 0.9923$). Analysis of subset mean concentrations, inclusive only of those steers yielding countable numbers of *Campylobacter*, revealed no effect of period, treatment group, or period \times treatment group interaction (Table 2).

The results from these studies do not support the hypotheses that distillers grain diets typically high in rumen-undegradable intake protein or supplemented with lasalocid will enhance intestinal carriage or shedding of *Campylobacter* in fed cattle. It is probable that, regardless of the protein source, feedlot diets containing 12% crude protein will provide nonlimiting amounts of amino acids capable of adequately supporting the growth of amino acid-metabolizing *Campylobacter* bacteria. For instance, Cecava et al. (4) reported duodenal flows exceeding 300 g of total amino acids per day (equivalent to as much as 78 mM) in cattle fed diets containing 12% crude protein provided as cotton seed meal, soybean meal, or corn gluten meal with urea, with much of the amino acid flow originating from microbial protein. Thus, we conclude that it is unlikely that feedlot practices of feeding rumen-undegradable intake protein or ionophores contribute to *Campylobacter* carriage or shedding.

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